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Serial No. 08/136,113

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of:
FINKEL, et al.

Serial No. 08/136,113 Art Unit 1805

Filed: October 13, 1993 Examiner Johnny F. Railey II

For: EFFICIENT AND SELECTIVE ADENOVIRAL-MEDIATED GENE TRANSFER INTO VASCULAR NEOINTIMA

APPEAL BRIEF

This brief is submitted, in triplicate, in the appeal of the above-identified application. A Notice of Appeal was filed January 11, 1996. A Table of Contents, a Table of Cases, a list of References Relied Upon, a copy of the Claims on Appeal , and a copy of a previously submitted and considered declaration of Dr. Toren Finkel, are attached as Appendices A-E, respectively. A request for a three month Extension of Time, to extend the due date to June 11, 1996, is enclosed herewith.

I. Real Party in Interest

The real party in interest is the government of the United States of America.

II. Status of Claims

The present application was originally filed, on October 13, 1993, with claims 1-17. In a February 9, 1995 amendment, new claims 18-21 were added. In an August 4, 1995 Office Action claims 1-21 were finally rejected. A February 12, 1996 Advisory Action indicated that all the pending claims remain rejected. Therefore, the claims on appeal are claims 1-21. A copy of the claims on appeal is attached herewith as Appendix D.

III. Status of Amendments

An amendment was filed on January 4, 1996 in response to the August 4, 1995 final rejection, which the Examiner indicated in the February 12, 1996 Advisory Action would be entered upon the filing of an appeal. This amendment included amendments to claims 15 and 16. The copy of the claims under appeal attached herewith as Appendix D incorporates this amendment to Claims 15 and 16.

IV. Summary of the Invention

Angioplasty failure rates of 25% to 50% within six months have been reported and confirmed by several authors. The smooth muscle cell (SMC) proliferation associated with arterial injury remains a major obstacle to the long-term success of coronary angioplasty. The injury activates medial SMCs, which begin to migrate and proliferate to form a neointima within the artery. Such neointimal cells are not present at the time of balloon injury. Animal models suggest that neointimal cells first begin to appear 3-5 days after vascular injury. The build up in an artery of neointimal cells is termed restenosis. The present invention is directed toward treating and/or preventing restenosis by transferring into neointimal cells a nucleic acid construct that can inhibit the growth of neointimal cells and thus treat restenosis.

Previous efforts to directly transduce arterial segments *in vivo* have used liposomal or retroviral methods to transfer marker genes into endothelial or SMCs, but the feasibility of such efforts, however, has been limited by a low transfection efficiency. In *in vivo* models, estimates of gene transfer into arterial segments range from fewer than 1 in 10,000 cells transduced with retroviral methods to fewer than 1 in 1,000 cells using liposomes.

Replication deficient recombinant adenoviral vectors have previously been shown to be efficient for transferring exogenous genes to a wide variety of cells *in vivo*. The genomes of such vectors can be manipulated so as to encode for recombinant gene products. The recombinant virus can be propagated in certain mammalian cell lines that serve to complement the growth of the replication defective adenovirus. Additionally, transduction by adenovirus, as opposed to retrovirus, does not depend on active replication of the host cell.

The present invention provides the surprising discovery that a nucleic acid can be targeted to neointima cells by using a replication-deficient recombinant adenovirus vehicle for gene transfer. Based upon this discovery, applicants have found that by administering the recombinant nucleic acid to the blood vessel as part of a recombinant adenoviral construct, the nucleic acid is targeted specifically to neointima cells. The method thus can be utilized to achieve selective expression of cytotoxic proteins and/or antisense constructs in neointima cells to inhibit growth of the neointima cells that the adenovirus selectively infects. Thus the development of neointimal formations can be hindered and existing neointima formations in the blood vessel can be reduced. In this manner, therefore, as shown by applicants, restenosis can be reduced and treated.

Specifically, the present invention provides a method of selectively expressing DNA in neointimal cells in an injured blood vessel of a subject comprising administering a replication-deficient recombinant adenovirus, which functionally encodes the DNA, to the blood vessel at the site of injury, such that the adenovirus remains at the site of injury for a time sufficient for the adenovirus to selectively infect neointimal cells and thereby selectively express the DNA in neointimal cells. Such methods are applied to treating restenosis by administering to the blood vessel an adenovirus which functionally encodes a DNA which can decrease the proliferation of neointimal cells.

V. Issues

Claims 1-21 stand rejected under 35 U.S.C. §112, first paragraph, claims 1, 5, and 6 stand rejected under 35 U.S.C. § 102(b), and claims 1-17 stand rejected under 35 U.S.C. § 103.

Regarding the rejection under 35 U.S.C. §112, first paragraph, the Advisory Action noted that applicants' January 4, 1996 amendment to claims 15 and 16 overcame the specific rejection of these claims regarding neointimal cell proliferation. Therefore, the issues remaining are (1) whether one of skill in the art would be able to practice the present method with any gene construct even though, as demonstrated by numerous citations herein, many such constructs are known in the art and the skilled artisan has the expertise to develop such constructs; and (2) whether applicants' claims that encompass use of the methods in human are properly enabled and described by the specification, even though applicants have utilized an animal model routinely used in the development of therapies in the art of restenosis.

Regarding the rejection under 35 U.S.C. § 102(b), the issue is whether or not the cited Willard *et al.* reference anticipates applicants methods of selectively expressing nucleic acids in neointimal cells when the Willard reference has not indicated that in their experiments neointimal cells were generated in the initially normal arteries they used.

Regarding the rejection under 35 U.S.C. § 103, the issue is whether a reference providing data indicating cells types (including neointima cells) that can be transfected using cationic liposomes, in combination with the above-described Willard reference, renders the present invention obvious, even though Willard that does not indicate if neointima formation was triggered and thus does not teach the transfection capabilities of adenovirus vectors on neointima cells, and even though liposome-mediated transfer is a different transfer method entirely.

VI. Grouping of Claims

The claims do not stand or fall together. Claims 1-7 and 17 are directed to methods of expressing DNA in neointimal cells and to a method of screening any selected DNA for ability to inhibit or decrease proliferation of neointimal cells. Claims 8-16 and 18-21 are directed to methods of decreasing proliferation of neointimal cells and treating restenosis and include claims using nucleic acids encoding a specific protein. The issues under 35 U.S.C. §112, first paragraph, do not apply in the same manner to each of these two sets of claims.

VII. Argument

A. Rejection under 35 U.S.C. §112, first paragraph, as applies to claims 1-7 and 17

Claims 1-7 and 17 are drawn to a method of selectively expressing DNA in neointimal cells in an injured blood vessel and to a method of screening DNA for the ability to inhibit or decrease proliferation of or to have cytotoxic effects on neointimal cells.

The Examiner's position

Claims 1-7 and 17 stand rejected under 35 U.S.C. §112, first paragraph, as the specification allegedly fails to provide an enabling disclosure and an adequate written description of the invention. The Examiner's points include the following, as set forth in the August 4, 1995 Office Action:

(1) To provide adequate support for method claims including gene constructs, applicants must provide adequate written description of these gene constructs, further noting that conception

of a DNA sequence requires adequate description of its chemical structure (citing *Fiers v. Sugano* 25 USPQ2d 1601 (Fed. Cir. 1993) and *Amgen v. Chugai,* 18 USPQ2d 1016 (Fed. Cir. 1991).

(2) Particularly with regard to the selection and expression of dominant negative genes and antisense constructs, the specification must provide an adequate written description of nucleic acid sequences that would function as claimed, alleging that it would require undue experimentation, with inadequate guidance provided by the specification, to determine gene constructs that would be useful in the invention, and alleging that there are essentially an infinite number of variations, including modifications, that would have to be tested to find a product fitting applicants' claims.

(3) The generation of any type of modified gene sequence and testing of the effect of the modification may not be undue if there is some guidance that limits the specific location and type of modifications, as well as involves a routine screening procedure, and allegedly the claims using dominant negative mutants and antisense RNA do not meet such criteria.

(4) The skilled artisan allegedly would have reason to doubt the correlation of applicants' data with use as an effective human therapeutic in the methods as claimed.

(5) Regarding claim 17, the specification allegedly fails to demonstrate any correlation between the DNA selected and efficacy in the screening procedure claimed, stating that a screening assay which suggests potential benefits of a therapeutic, which benefit never materialized in practice, is indicative of a fundamental problem with the screening assay as a predictor.

Applicants' Resp nse

(1) Regarding support for claims including gene constructs and the requirement to provide adequate written description of these gene constructs, applicants note that claims 1-7 and 17 are drawn to methods of expressing DNA in neointimal cells, and the presence of teachings and examples of expression of some gene constructs, along with guidance as to other constructs, is sufficient to enable these claims. Regarding specific constructs, the only claims specifying any particular kind of gene construct are claims 6 and 7, and if this point of rejection is to be maintained, it should be made clear to which claims it is directed.

The claims are directed to methods in which any desired gene expressible in a mammalian system can be used, that is, a generic method of expressing genes in neointimal cells in an injured blood vessel. By applicants' discovery and demonstration that a nucleic acid can be expressed in neointimal cells in an injured blood vessel by placing the nucleic acid in a replication-deficient adenovirus and administering the recombinant adenovirus to the blood vessel, they are entitled to claims of the scope provided in this application. The core discovery allows the expression of <u>any</u> desired gene in neointimal cells. Therefore, conception of particular genes is simply not an issue for enabling these claims.

Additionally, however, nucleic acids encoding the specific sequences (proteins or antisense RNA) specified in the method claims 6 and 7 are <u>known</u> and published and, in fact, the nucleotide sequence of the specifed genes themselves have been elucidated and are part of the art at the time of filing this application. In support of this statement, applicants cite the following references, which are in the record, copies of which having been previously provided in applicants' Information Disclosure Statement. These references disclose sequences for antisense nucleic acids as indicated: Biro, *et al. Proc. Natl. Acad. Sci. USA* 90:654-658 (1993) (c-myc); Simons, *et al. Nature* 359:67-70 (1992) (c-myb); Speir, *et al. Circulation* 86:538-547 (1992) (PCNA). These references in fact are cited in the specification, though such citation in the specification is

not required in order to support this point. Therefore, nucleic acids encoding these specific cytotoxic molecules have been conceived, and these nucleic acids are known in the art.

That which is known in the art is preferably omitted in a patent application (*Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F2d. 1367, 231 USPQ 81, 94 (Fed. Cir. 1986)). The specification is addressed to the worker skilled in the art, so details known to that worker need not be included. *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 221 USPQ 481 (CAFC 1984). This issue was more fully addressed in *In re Chilowsky*, 108 USPQ 321 (CCPA 1956), where the Court stated:

> It is well settled that the disclosure of an application embraces not only what is expressly set forth in words or drawings, but what would be understood by persons skilled in the art. As was said in *Webster Loom Co. v. Higgens et al.*, 105 U.S. 580, 586, the applicant "may begin at the point where his invention begins, and describe what he has made that is new and what it replaces of the old. That which is common and well known is as if it were written out in the patent and delineated in the drawings."

Thus, the nucleotide sequence of these nucleic acids need not be recited in the specification in order for claims utilizing them to be enabled.

Furthermore, regarding the Examiner's point (2) that there are essentially an infinite number of variations of these genes, including modifications, that would have to be tested to find a product fitting applicants' claim, applicants note that to enable a method claim utilizing nucleic acids constructs, applicants need not recite specifically every useful gene construct; applicants need only teach how to perform the method and provide guidance for selecting additional genes,

which applicants clearly do, as described herein. Applicants provide examples as well as teachings as to additional genes.

On this point, in *In re Anderson*, 176 USPQ 331 (CCPA 1973), the Court stated:

> We do not regard Section 112, first paragraph as requiring a specific example of everything within the scope of a broad claim. *In re Gay*, 50 CCPA 725, 309 F.2d 769, 135 USPQ 311 (1962). There is no question raised as to the fact that there are specific examples of what appears to be the preferred embodiment and best mode contemplated by the applicant of carrying out his claimed invention; we are here dealing only with a possible alternative embodiment within the scope of the claims. What the Patent Office is here apparently attempting is to limit all claims to the specific examples, notwithstanding the clear disclosure of a broader invention. This it may not do.

Such is the present situation. Applicants' claims 1-7, are directed generally to methods of expressing DNA in neointimal cells, and applicants' core discovery allows them to claim broadly the expression of genes in neointimal cells. In addition to published, known gene constructs, applicants have provided teachings in the specification, such as at page 5, line 30-page 6, line 28, and in the examples, regarding making such constructs, and persons skilled in the art are fully capable of preparing expression constructs for genes of interest, particularly in light of the above-cited teachings. Applicants have additionally provided sufficient teachings, such as at page 4, lines 23-31, page 5, lines 1-11 and in the examples, regarding using the claimed method to express genes in neointimal cells. As cited below, that which is known in the art is preferably omitted in an application, and constructing multiple additional expression vectors for a gene of interest falls within that which is known in the art, particularly in light of applicants' teachings. Therefore, clearly, Claims 1-7 are described and enabled by the specification.

Specifically, addressing the comments regarding antisense RNA, in addition to those sequences known in the art, applicants have provided specific teachings regarding antisense constructs at page 6, lines 25-29 and page 7, lines 7-9. Additionally, both the *Spier, et al.* and *Biro, et al.* references cited in the specification provide several examples of oligonucleotides that demonstrate success as antisense molecules, indicating that significant guidance has been provided by the specification and that one skilled in the art certainly can routinely generate and optimize additional antisense molecules for use in the present invention, given applicants' teachings. Furthermore, regarding potential varying levels of usefulness of additional antisense constructs, there is no requirement that all of the molecules in a claimed invention exhibit the same degree of efficacy in order to meet the requirements of 35 U.S.C. §112, first paragraph. *In re Gardner*, 177 USPQ 396 (CCPA 1973).

Regarding point (3) that the generation of any type of modified gene sequence and testing of the effect of the modification may not be undue if there is some guidance that limits the specific location and type of modifications, as well as involves a routine screening procedure, but that allegedly the claims using dominant negative mutants and antisense RNA do not meet such criteria, applicants believe this point has been addressed fully above in discussing the information in the literature regarding specific nucleotide sequences for such constructs, in discussing the skill of the artisan in making modifications as desired, and in discussing the position of the courts regarding a lack of a requirement for teaching any specific embodiment when a general method is claimed.

Additionally, however, applicants have provided a screening procedure that can routinely be performed for any selected nucleic acid to determine potential usefulness of the nucleic acid. It is a simple matter of substituting the gene of choice for the gene used by applicants in the example. In particular, examples are provided of *in vitro* experiments with transfer of adenoviral-

gene constructs into vascular smooth muscle cells and of *in vivo* transfer into injured arterial segments which teach methods by which one can routinely screen a gene for cytotoxicity. Furthermore, the examples, wherein the β-galactosidase gene, the CFTR gene and the thymidine kinase gene are successfully transferred into neointima cells provide specific guidance for the transfer which applies fully to transfer of any other gene. It can hardly be argued that this simple substitution constitutes undue experimentation.

Regarding the point (4) that the skilled artisan allegedly would have reason to doubt the correlation of applicants' data with use as an effective human therapeutic in the methods as claimed, applicants note that these claims are simply for expressing a nucleic acid, rather than claiming any particular therapeutic result. However, the Examiner has previously alleged that although claims 1-7 are directed to the *in vivo* expression, there is no disclosure of how to use this method other than in a therapeutic context. Therefore, applicants will address this point for claims 1-7 and 17.

First, certainly there is indeed utility in this expression for screening nucleic acids for the ability to decrease proliferation of neointimal cells. Such a utility is completely sufficient. However, additionally, the specification teaches that one can express a protein in neointimal cells. Thus many other genes can be expressed in neointimal cells, for any desired purpose that the skilled artisan may have, such as the provision of a protein. Having established that indeed there is utility in the expression and the screening of nucleic acids as claimed in claims 1-7 and 17, and that applicants' data is clearly sufficient for claims to expressing and screening a nucleic acid, this rejection no longer applies to claims 1-7 and 17.

Finally, regarding Examiner's point (5) specifically regarding claim 17, and alleging that the specification fails to demonstrate any correlation between the DNA selected and efficacy in the screening procedure claimed, concluding that a screening assay which suggests potential

benefits of a therapeutic, which benefit never materialized in practice, is indicative of a fundamental problem with the screening assay as a predictor.

Claim 17 is drawn to a method of screening DNA for "the ability to inhibit or decrease proliferation of or to have cytotoxic effects on neointimal cells." Clearly applicants have shown in their examples that the claimed screening method indicated that the thymidine kinase gene, in its regimen with ganciclovir, has "the ability to inhibit or decrease proliferation of or to have cytotoxic effects on neointimal cells." The adenoviral vector alone, which encodes certain non-inhibitory adenoviral genes, did not have an effect on proliferation or cytotoxicity. Additionally, the adenoviral construct encoding CFTR, which is not a construct expected to inhibit neointimal cells, was shown by the screening method not to inhibit neointimal cells. Thus, contrary to the conclusion of the Examiner, applicants have shown that the screening method does credibly have the capability of screening out some genes that do not inhibit or decrease proliferation of or have cytotoxic effects on neointimal cells.

Further, it has clearly been shown that the method has the capability of screening out some genes that would have no therapeutic potential. Thus, by showing that the screening method does have the capability of screening out some genes that do not inhibit or decrease proliferation of or have cytotoxic effects on neointimal cells and does have the capability of screening out some genes that therefore would have no therapeutic potential, correlation between the DNA selected and efficacy in the screening procedure claimed has been shown.

Furthermore, in response to the specific allegations of the examiner in the August 4, 1995 Office Action, that there is nothing in the specification to teach the skilled artisan which DNA sequences, when expressed, would be predictive as an effective human therapeutic, applicants contend that is the purpose of the screening method of claim 17. Thus, the screening method itself clearly would indicate to the artisan which DNA sequences, when expressed, would be

predictive as a potential therapeutic. That is, those DNA sequences which when screened, as recited in claim 17, are found to inhibit or decrease proliferation of or are cytotoxic to the neointimal cells formed in the injured blood vessel of the assay are DNA sequences predictive as an effective human therapeutic. The specification also provides a clear example of a sequence which, when expressed and screened, had potential as a therapeutic, namely the herpes virus thymidine kinase (tk) gene.

Applicants note that this method is a screening method, not an absolute predictor, and even if it indicates a DNA to have potential therapeutic effectiveness, and that DNA is ultimately determined not to have significant therapeutic benefit, the screen was still useful in narrowing down the focus of which genes to further pursue by ruling out some genes which show no potential for benefit in the method. Applicants are aware of no general rule that to be an acceptable screening assay can never suggest a potential benefit of a particular therapeutic, which benefit never materializes in practice. The present screening method, whether or not some genes indicated by the procedure to have potential for therapeutic use ultimately are found not to have such a use, satisfies the statutory requirements of 35 U.S.C. §112, first paragraph, by its usefulness in narrowing the focus of potentially therapeutic constructs, which use is fully enabled.

B. Rejection under 35 U.S.C. §112, first paragraph, as applies to claims 8-16 and 18-21

Claims 8-16 and 18-21 are directed to methods of decreasing proliferation of neointimal cells and treating restenosis and include claims using nucleic acids encoding a specific protein.

Examiner's position

Claims 8-16 and 18-21 stand rejected under 35 U.S.C. §112, first paragraph, as the specification allegedly fails to provide an enabling disclosure and an adequate written description

of the invention. The examiner has alleged the above described points (1)- (4) as to claims 8-16 and 18-21.

Applicants' Response

1. As regards points (1) -(3), applicants' above comments apply and fully address this rejection as it also is applied to claims 8-16 and 18-21. A few additional comments follow.

Regarding points (1)-(3), in addition to the comments above, applicants note that the analysis in the Office Action ultimately concludes that the scope of protection sought by applicants fails to bear a reasonable correlation to the scope of enablement provided by the specification, and that applicants have not taught one of skill in the art how to make and use the full scope of the invention without undue experimentation, pointing particularly to the various gene constructs one can use in the present invention as claimed. The Office Action admits, however, that the generation of any type of modified gene sequence and testing of the effect of the modification may not be undue if there is some guidance that limits the specific location and type of modifications, as well as involves a routine screening procedure.

In the present case, the claimed sequences are known in the art to be cytotoxic or inhibitory to cellular proliferation; therefore, it is indeed credible that they would function to inhibit or decrease proliferation of neointimal cells into which they are transfected and thus treat restenosis. A specification need not contain, and preferably omits, that which is known in the art. *Hybritech Inc. v. Monoclonal Antibodies, Inc.* 231 USPQ 81, 94 (Fed. Cir. 1986), *cert. denied,* 480 U.S. 947 (1987). The art cited in the specification provides details characterizing the specific constructs of selected nucleic acids found by the art to express well. Furthermore, as described below, the skilled artisan has thorough knowledge of designing cytotoxic and/or proliferation-inhibitory gene constructs. That this is credible evidence to support these claims is clearly

supported by the example provided in the specification wherein the regimen including selective expression of thymidine kinase in combination with treatment with ganciclovir was shown to be effective in treating restenosis. This shows a credible correlation between genes/regimens shown to be cytotoxic and/or cause a decrease in neointimal cell proliferation and their use in the present method to inhibit or decrease proliferation of neointimal cells or to treat restenosis.

Regarding whether the generation and testing of modifications to these gene sequences can be considered to be undue experimentation, applicants contend that it is not undue, as described below. It is well settled that it is not merely the amount of experimentation that determines whether it is undue, since a considerable amount is permissible if it is merely routine or if the specification provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988). In the present application, in sum, simply substituting one gene for another in an assay cannot be considered undue experimentation. Specifically, given the knowledge of the art of vector construction and testing for cytotoxic effects of a gene or antisense construct, to perform the claimed method is not undue experimentation.

Ex parte Forman, 230 USPQ 546 (Bd. App. 1986), applied in *Wands*, set forth the criteria for establishing whether obtaining a claimed invention requires undue experimentation. The factors to be examined to determine whether experimentation is undue are: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

Applying these factors to the present claims, (1) the quantity of experimentation is quite low since one must simply substitute one gene construct for another and perform the screening

method that is thoroughly described in the specification. Once the screen has been performed, it is clear whether or not a given construct screens positively for inhibiting or reducing neointimal cells. Regarding modifications to known gene sequences, again the quantity of experimentation is low because guidelines for selecting various modifications are well-known in the art and any selected modified gene construct is then simply run through applicants' screening procedure. Furthermore, in choosing a gene to modify in a desired manner, knowledge regarding nucleic acids encoding cytotoxic or proliferation-inhibitory proteins and antisense RNA is in the art (as exemplified by Biro (c-myc); Simons (c-myb); Speir (PCNA), all cited above; Epstein *et al.* *Circulation* 84:778-787 (1991) (*Pseudomonas* endotoxin), all references having been cited in the specification). Thus the quantity of experimentation is very low.

(2) There is a significant amount of direction or guidance presented by the specification for expressing selected gene constructs in smooth muscle cells *in vitro* and in neointimal cells *in vivo,* such as at page 4, line 23- page 5, line 11; page 7, lines 17-27 as well as by the detailed examples. Additionally, the specification provides significant guidance regarding choosing and constructing gene constructs, such as at page 6, lines 1-5; page 6, lines 7-20; page 6, lines 22-28; page 7, lines 5-15, and in particular cites several examples of useful nucleic acids that can be used as disclosed or modified in a desired manner (*e.g.*, page 7, lines 10-15).

(3) The present specification provides numerous working examples, which examples provide teachings of how to perform the methods that can readily be applied to any selected construct. For example, page 9, line 3-page 10, line 7 and page 13, lines 3-14 provide working examples of *in vitro* expression of gene constructs; page 10, line 9- page 12, line 30 and page 13, line 14- page 18, line 21 provides working examples of *in vivo* expression of gene constructs (β-gal and thymidine kinase) and working examples of inhibition of neointimal cells (using thymidine kinase expression and ganciclovir treatment). These working examples provide inhibition of

neointimal cells in a mammalian model which has been used extensively in the literature for restenosis testing.

(4) The nature of the invention is such that any gene construct can readily be made according to well-known techniques and theories for modifications and screened by following applicants' clear teachings. Thus any selected construct can readily be screened for usefulness to treat restenosis.

(5) The state of the prior art is such that the art of gene construction is very well-known and additional gene constructs can therefore be routinely made. Additionally, the nucleic acid sequences of the suggested useful genes as well as many others indicated in the art to be useful in inhibiting cell proliferation, are known, and many antisense constructs have also been developed. Furthermore, in combination with applicants' teachings, the art in animal models, particularly in administration of compounds to blood vessels, is such that a skilled artisan is provided the guidance needed to readily perform the screening procedure taught and exemplified by applicants.

(6) The relative skill of those in the art is high, with the average artisan being the level of a Ph.D. An artisan of that level is well versed in the construction of gene constructs, as well as in the modification of such constructs and the guiding principles discovered over the years for different modifications. Such an artisan is also well versed in the literature concerning genes that inhibit proliferation of cells and antisense constructs. Thus, persons of skill in the art know the parameters to be considered and presumably expect when working in the field of antisense technology to consider these parameters when selecting a particular construct.

(7) The predictability of the art is such that one can readily predict the successful construction of a desired construct and that one can successfully test the construct in the present

screening method. The examples in the specification demonstrate that a construct previously known to inhibit the proliferation of cells, when used in applicants' method, displayed this same inhibitory characteristic. Furthermore, a construct lacking a known inhibitory gene was demonstrated in the examples not to inhibit proliferation. Therefore, one can, with fairly accurate predictability, predict that gene products known in the art to inhibit cell proliferation are good candidates for the present screening method, and upon success in the screen, are candidates for use in treating restenosis.

(8) The claims are limited to expression in neointimal cells, and success with neointimal cells is clearly provided in the specification in the examples. Furthermore, the claims are limited to use of a replication-deficient recombinant adenoviral vector that functionally encodes the gene product. Use of this recombinant adenoviral vector is fully taught in the specification, including the many examples utilizing it. Thus, the claims, with the exception of the specific gene construct are limited to that exemplified. The choice of gene construct, however, is well within the skill of the ordinarily skilled artisan, much information regarding useful cell proliferation inhibitors is in the literature, and specific examples of inhibition are provided in the specification. Thus, clearly, the skilled artisan has been taught how to make and use the present invention.

Furthermore, while not necessary in view of the examples, applicants previously provided a declaration of Dr. Toren Finkel, a skilled artisan, provided herewith as Appendix E. The February 5, 1996, Advisory Action indicated that this declaration had been considered, but does not place the application in condition for allowance, with no specific remarks regarding the comments of Dr. Finkel. Applicants believe the comments of Dr. Finkel should be given full weight.

This declaration notes that the specified genes in the method claims are cytotoxic or inhibitory to cell proliferation, and that therefore, it is more likely than not that such genes

transfected into neointimal cells would be cytotoxic and/or cause a decrease in neointimal cell proliferation. This conclusion is further based on the example set forth in the specification. The declaration further states that, because the other recited nucleic acids (in addition to thymidine kinase plus ganciclovir) would likely be cytotoxic and/or cause a decrease in neointimal cell proliferation, they would also likely treat restenosis. The Examiner has not specifically addressed this declaration, which contradicts the assumptions alleged in the Office Action.

Applicants contend that this declaration provides significant support for applicants' contention, fully supported by other literature cited above, that other gene constructs can readily be selected for high probability of success in use in the present method to decrease or inhibit neointimal cell proliferation and treat restenosis, and can then simply be screened and ultimately used for decreasing or inhibiting neointimal cell proliferation according to the teachings in the specification. Therefore, the present application fully teaches the skilled artisan how to make and use the present invention.

2. Regarding claims 8-16 and 18-21, the Examiner has further alleged that the art supports a conclusion that the skilled artisan would have reason to doubt the correlation of applicants' data in rats with use as an effective human therapeutic in the methods as claimed. Applicants first note that the standard applicable in this rejection is whether applicants have presented credible evidence that the method will work, not whether there is reason to doubt that it will work. Applicants, in a February 9, 1995 amendment, cited Schwartz, *et al.* (U.S. Patent No. 5,304,122) as evidence that credibility is given generally to animal models (in Schwartz, a porcine model) by skilled artisans. The August 4, 1995 Office Action rebutted this point by alleging that Schwartz does not disclose or claim methods using human therapeutics by their procedure.

However, Schwartz clearly provides evidence of credibility given to animal models, and Schwartz clearly states in the abstract that their porcine model "closely mimics the proliferative

portion of human restenosis both grossly and microscopically" and at column 13-14, it states that "[t]his porcine model for the proliferative component of human restenosis is accurate....Therapies aimed at reducing the occurrence of restenosis might thus easily be evaluated using this model."

The August 4, 1995 Office Action additionally pointed to *Ohno* et al., previously provided by applicants, to support that rat models in particular may be considered to be uncertain. However, clearly many skilled artisans utilize the rat model for restenosis testing. For example, the above-cited Speir, Simons, and Biro references, as well as *Casscells et al, Proc. Natl. Acad. Sci. USA* 89: 7159-7163 (1992), cited in the specification, use a rat model. Therefore, there is credible correlation between the rat model studies provided by applicants and their claimed methods.

Additionally, arguments presented in 3. below regarding claims 18-21 and the use of the present methods in humans based on data from animal models fully apply to claims 8-16.

3. Regarding claims 18-21, applicants believe these claims should have been considered separately under this 35 U.S.C. §112, first paragraph, rejection. These claims are limited specifically to the known herpes virus thymidine kinase gene and to reducing, rather than preventing, neointimal cell proliferation. Data is provided in the specification (page 15, line 27; page 16, line 25) demonstrating its use. Applicants have thus demonstrated reducing neointimal cell proliferation as recited by the claims. The August 4, 1995 Office Action stated that claims 18-21 are free of the art. Therefore, the only point of the above rejection that can possibly apply to these claims would be that a human use has not been demonstrated. However, the PTO has clearly stated :

> If reasonably correlated to the particular therapeutic or pharmacological utility, data generated using *in vitro* assays or from testing in an animal model or a combination thereof almost invariably will be sufficient to establish therapeutic or

> pharmacological utility for a compound, composition or process. In *no* case has a Federal court required an applicant to support an asserted utility with data from human clinical trials. *(emphasis in original)*

PTO Examination Guidelines on Utility Requirement, page 306.

While the above quotation is excerpted from guidelines regarding utility, the issue here is enablement of the human *in vivo* utility that is asserted by virtue of the claims reading on animals other than just the rat used in the examples. Applicants, by their examples, have clearly taught how to perform the methods in humans- one simply follows the example. The issue is its believability to work in humans, which the Patent Office is addressing in the above paragraph. Thus the above PTO guideline applies in this analysis.

The Patent Office continues, at page 307, to state that if data, whether *in vitro* or animal tests or both, and an explanation of why that data supports the asserted utility, is provided, the Patent Office will determine if the data and the explanation would be viewed by one skilled in the art as reasonably predictive of the asserted utility. In the present application, applicants have not only provided both *in vitro* and animal model data, they have also provided citations to many current literature references wherein the rat model of restenosis has been utilized, and continues to be utilized, by respected scientists in the field.

Therefore, applicants believe that claims 18-21 clearly are allowable.

4. Specifically regarding Claim 16, drawn to methods of treating atherosclerosis, the August 4, 1995 Office Action alleged that it is not evident from applicants' disclosure that atherosclerosis has been treated, and that the two conditions of atherosclerosis and neointimal cell proliferation are not commensurate. Further, the Office Action alleged that the level of cell proliferation in neointimal formation associated with primary atherosclerosis is not shown to be equal to that

associated with balloon injury, and that, consequently, achieving an effective level of expression of tk to decrease neointimal formation in atherosclerosis is not, without evidence to the contrary, directly correlated or predictive.

Applicants contend that proliferation of neointimal cells is known to play a significant role in the progression of atherosclerosis. First, the August 4, 1995 Office Action itself argued, at page 12-13, that the cited Takeshita (abstract 3179) reference shows that there is equally efficient gene transfer and expression *in vivo* for percutaneous translumenal angioplasty (PTA) versus non-PTA treatment prior to liposome-mediated plasmid transfection of atherosclerotic iliac arteries.

Additionally, provided with applicants' January 4, 1996 amendment was a review article, Ross, R. "The Pathogenesis of Atherosclerosis: a perspective for the 1990s", *Nature* 362:801-809 (1993). Ross states at page 803, column 1, that three processes are involved in the formation of atherosclerotic lesions: (1) the proliferation of smooth muscle cells; (2) the formation by smooth muscle cells of a connective tissue matrix comprising elastic fibre proteins, collagen and proteoglycans; and (3) the accumulation of lipid and mostly free and esterified cholesterol in the surrounding matrix and the associated cells. Ross continues on to discuss how proliferation and/or migration of smooth muscle cells plays a role in the progression of atherosclerotic lesions (esp. page 803, column 1 to page 804, column 1). Further, Ross indicates, at page 808, column 1 that cell migration and proliferation are critical events in both restenosis and the primary atherosclerotic lesions. Finally, Ross concludes that development of specific means of selective inhibition of each of the events that contribute to the advanced lesions of atherosclerosis should provide opportunities to diagnose, treat and prevent the process of atherosclerosis.

Furthermore, provided with applicants' January 4, 1996 amendment was Flugelman *et al.*, *Circulation* 88(6):2493-2500 (1993), which shows that histologically and immunochemically the lesions of unstable angina pectoris is similar to that of the restenotic lesion.

Therefore, it is known in the art that the proliferation of smooth muscle cells plays a key role in the formation and progression of atherosclerotic lesions. Thus, contrary to the allegations in the Office Action, achieving an effective level of tk to decrease neointimal formation is correlated with treating atherosclerosis.

C. Rejection under 35 U.S.C. §102(b)

Examiner's position

Claims 1, 5 and 6 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Willard *et al.*, as previously rejected.

Applicants' Response

Willard, however, does not disclose neointimal formation or cellular proliferation at all and therefore in no way teaches selective expression in neointimal cells, as applicants have claimed. Willard's experiments importantly are performed in normal veins and arteries. Furthermore, Willard merely teaches that certain cell layers can be targeted for gene transfer by physical placement of the adenoviral vector. That is, (1) they achieve expression in the endothelium in veins in which the adenovirus was injected into the lumen and allowed to dwell for a time period, and (2) they achieve expression at the site of infusion ("the site of medial disruption") when the adenovirus is infused into an artery wall by high velocity jets (not normally used to infuse drugs), which jets physically disrupt the artery wall, thereby physically causing the placement of the gene into the medial layer. These two demonstrations simply do not teach selective expression of DNA in neointimal cells by merely contacting the replication-deficient adenovirus with the injured blood vessel, in particular since in Willard no neointimal cells are present. As stated in Willard, this discloses only that adenovirus "can be preferentially directed at specific layers of the vessel wall." Therefore, the present invention, which claims a method of selectively expressing DNA in neointimal cells, is not anticipated by Willard.

The Advisory Action states that Willard does teach balloon catheter infusions, not just infusion by high velocity jets, and that, absent evidence to the contrary, this references discloses applicants' methods of gene transfer and expression, regardless of the fact that applicants characterize their invention as gene transfer to "neointimal cells."

Applicants contend, however, that without teaching that their catheter infusion triggered a neointimal response, there is no teaching in Willard that one can selectively express a gene in neointimal cells. The Examiner is arguing, in essence, that Willard inherently disclosed applicants' method of selectively targeting neointimal cells. However, there is nothing in the record to indicate that inherently Willard did transfect neointimal cells. Neointimal cells are only formed when sufficient injury occurs to activate smooth muscle cells, and Willard simply does not indicate that neointimal cells were formed. Furthermore, Willard did not indicate any cell type transfected; the reference merely says expression was limited to the "site of medial disruption." Thus it is not clear, for example, whether medial smooth muscle cells or adventitial fibroblasts were infected. It is only with impermissible hindsight in light of applicants' disclosure that one can allege that Willard must have expressed their gene in neointimal cells, and there is no indication that Willard caused neointimal formation.

Contrary to the statement in the Advisory Action, this is not merely a matter of words selected to characterize the transfected cells. Applicants' method is directed to selectively expressing DNA in neointimal cells, and Willard does not teach such expression, regardless of a teaching of expression limited to the site of medial disruption. As stated above, without a further teaching that their use of the catheter triggered a neointimal response, this reference simply does not disclose expression in neointimal cells. Not all balloon catheters cause neointima formation, and not all methods of using balloon catheters cause a neointima response. Therefore, the mere fact of Willard using a balloon catheter does not teach neointima formation.

D. Rejection under 35 U.S.C. § 103

1. **Examiner's position**

Claims 2-4 stand rejected under 35 U.S.C. § 103 as allegedly obvious over Willard *et al.* as applied to Claims 1, 5 and 6 and further in view of Takeshita *et al.* (abstract 0903). Specifically, the Office Action alleged that Takeshita, with experiments using a cationic liposome to deliver genes, teaches that gene transfer, *i.e.*, uptake and expression of nucleic acid sequences, is enhanced in general when cells are actively proliferating. Further, the Office Action alleged that the skilled artisan would have recognized that increased gene uptake, regardless of whether it is delivered by liposomes or adenovirus vector, would correlate with increased availability of gene sequences for expression in the cell, and the fact that adenoviral vectors do not require host cell division for gene expression has no bearing on the effect of uptake and expression of nucleic acids by dividing cells. The Office Action concluded that, as allegedly shown by Takeshita, it would have been apparent to the skilled artisan that active host cell division enhances uptake and expression of exogenous nucleic acid sequences.

Applicants' Response

Applicants' claims are to selectively expressing DNA in neointimal cells in an injured blood vessel. Neither Takeshita nor Willard provide teachings regarding expression in neointimal cells, nor do they suggest a method of obtaining expression in neointimal cells. Takeshita allegedly teaches that gene transfer (uptake and expression) by liposomes is enhanced when cells are actively proliferating. This teaching does not suggest, without anything further, that neointimal cells can be selectively transfected by use of adenoviral vectors. This assertion by applicants is supported by O'Brien, E.R.. *et al.*, *Hypertension* 20(6):713-736 (Dec. 1992), the abstract of which was provided with applicants' January 4, 1996 amendment, which indicates that in human restenotic arterial lesions, only modest numbers of SMCs, macrophages and endothelial cells proliferate. Thus, Takeshita's conclusions regarding expression in actively proliferating cells do

not suggest, and therefore do not render obvious, applicants' methods of selectively expressing DNA in neointimal cells.

Additionally, applicants note that Willard states that, compared to vein segments, arterial wall gene transfer was "less efficient." As such, there is no way of knowing whether adenoviral gene transfer is in any way superior to previously described methods for arterial gene transfer. Thus there is no motivation provided by Willard to combine its method with any other to attempt to achieve superior arterial transfection results.

As described in C. above, Willard does not teach expression in neointimal cells. Therefore, this obviousness rejection is at best an "obvious to try" rejection to see if expression in neointimal cells can be obtained, which is clearly improper.

Furthermore, the Office Action, as stated above, states that "the skilled artisan would have recognized that increased gene uptake, regardless of whether it is delivered by liposomes or adenovirus vector, would correlate with increased availability of gene sequences for expression in the cell." (emphasis added). This analysis clearly misses the inventiveness of the present method. Nowhere has it been stated or is it taught or suggested in these references that uptake of a gene in an adenoviral vector can be made selective for neointimal cells. Applicants' discovery therefore was unexpected.

Specifically, regarding the alleged link between cell proliferation and increased gene transfer, the art does not reflect any clear link between cell proliferation and uptake of adenoviral vectors. The art at best can be argued to reflect a link between proliferating cells and transfer via the liposomes used in this reference. The art does not demonstrate a generic link between all proliferating cells and adenoviral vectors.

By way of support for this stance, applicants cite the following literature references that show use of adenoviral vectors to transfect non-proliferating cells: In Guzman, *et al, Circulation Research* 73: 1202-1207 (1993), provided with applicants' January 4, 1996 amendment, adenoviral vectors are shown to transfer genes into myocardium, which are non-proliferating cells, with high efficiency. In Quantin *et al*, cited in the Office Action, adenoviral vectors are shown to transfer genes into myotubes and muscle.

Further, to support applicants' stance, applicants cite the following literature reference that shows that adenoviral vectors have demonstrated low transfection efficiencies in some proliferating cells: Huang, S. *et al., J. Virol.* 69(4):2257-2263 (1995) , provided with applicants' January 4, 1996 amendment, shows gene transfer with adenoviral vectors into lymphocytes, which are actively proliferating cells, to have low efficiency.

Thus, clearly the literature does not support the Examiner's allegation that gene transfer by adenoviral vectors, *i.e.*, uptake and expression of nucleic acid sequences, generically is enhanced when cells are actively proliferating. The above-cited references refute such an general allegation. Thus, clearly, prior to applicants' invention, the skilled artisan would not have known that one could achieve selective transfer, and thus selective expression, of genes into neointimal cells with adenoviral vectors, and could not have known that optimal time parameters for infection would be days 4-15 post-injury. And thus, the teachings of Takeshita, regarding uptake and expression of genes transferred via liposomes, are not relevant to applicants' discovery.

Furthermore, applicants contend that the Office Action assumes that one method of gene transfer can readily be substituted for another in a given method to obtain the same results without providing any basis for such an assumption. The two transfer methods, a cationic

liposome-mediated transfer and an adenovirus-mediated transfer, and cannot be considered equivalent, since they present to a cell two different compositions. Additionally, though the mechanisms of neither method are fully elucidated yet, it is known that many cells express adenoviral receptors on their surface by which transfer of genes in adenoviral vectors may be accomplished. Liposome-mediated transfer, on the other hand, does not appear to be receptor-specific, and particularly does not appear to be specific for cells with adenoviral receptors. Thus, there is no basis that has been presented for assuming that the two methods are equivalent in transfer into the cell. And applicants, above, show that indeed one cannot generally assume that adenoviral vectors transfer well into actively proliferating cells.

Therefore, applicants contend that claims 2-4 are not obvious over the cited combination of references.

2. **Examiner's position**

Claim 17 stands rejected under 35 U.S.C. § 103 as allegedly obvious over Schwartz *et al.* in view of Willard *et al.*, the Office Action alleging that Willard teach essentially the same methods as applicants using adenoviral methods, that is, methods of selectively infecting neointimal cells, and that Schwartz discloses essentially the same diagnostic method as recited in claim 17 but without the adenoviral vector.

Applicants' Response

Claim 17 recites a method of screening DNA for the ability to inhibit or decrease proliferation of or to have cytotoxic effects on neointimal cells, comprising administering to an injured blood vessel in a subject at the site of injury a replication-deficient adenovirus which functionally encodes the DNA, for a time sufficient for the adenovirus to selectively infect neointimal cells; and detecting inhibition or decrease of proliferation of or toxicity to the neointimal cells, such inhibition or toxicity indicating a DNA having the ability to inhibit or

decrease proliferation of or to have cytotoxic effects on neointimal cells. Applicants assert that the use of an adenoviral vector to selectively infect neointimal cells is not taught in the combination of cited references.

Willard does not teach transfer into neointimal cells; thus they could not teach selective transfer into neointimal cells. As stated above, Willard discloses only that adenovirus "can be preferentially directed at specific layers of the vessel wall" by virtue of infusion into the wall at the site of infusion. It is only with impermissible hindsight that one can conclude that Willard taught selective expression of neointimal cells. Therefore, the combination of Willard with the diagnostic method of Schwartz does not teach or suggest applicants' method of screening, because the combination does not teach or suggest selective expression in neointimal cells with an adenoviral vector. Thus, applicants' screening method is not rendered obvious by this combination of references.

3. **Examiner's position**

Claims 1, 5, 6, 8, 10 and 15-17 stand rejected under 35 U.S.C. § 103 as allegedly obvious over Takeshita *et al.* (3179) in view of Quantin *et al.* or Willard *et al.* In response to applicants' previous arguments that Takeshita's teachings that liposome-mediated gene transfer is not affected by angioplasty teach away from a method wherein neointimal cells are selectively targeted, the August 4, 1995 Office Action alleged that Takeshita simply shows that there is equally efficient gene transfer and expression *in vivo* for angioplasty versus non-angioplasty treatment of atherosclerotic arteries prior to gene transfer via liposomes.

Applicants' Response

Applicants contend that neither Willard, Quantin, nor Takeshita teach selective transfer into neointimal cells, and thus the present invention has not been taught by this combination of references. As noted by the Examiner, Takeshita teaches that there is equal efficiency of gene

transfer via liposomes in atherosclerotic arteries before and after angioplasty treatment. Takeshita's conclusion is merely that angioplasty does not alter the transfection efficiency of liposome-mediated transfer in atherosclerotic arteries. Takeshita in no way teaches or suggests the selective transfection of neointimal cells, by any means of transfer. And in particular, it also does not teach or suggest the use of adenoviral-mediated gene transfer or in any way suggest that one can achieve selective transfection of neointimal cells by adenoviral-mediated gene transfer. Furthermore, efficiency of delivery and expression of luciferase with liposomes by Takeshita was low (11.7 Turner light units in PTA-treated lesions and 35.8 Turner light units in non-PTA treated lesions).

Willard, as stated above, discloses only that adenovirus "can be preferentially directed at specific layers of the vessel wall" by virtue of infusion into the wall at the site of infusion. Willard does not indicate whether their catheter infusion triggered a neointimal response. Willard thus in no way teaches or suggests selective expression in neointimal cells, and any combination of references with Willard to conclude that one can achieve selective expression in neointimal cells (wherein none of the references disclose transfer into neointimal cells with adenovirus) could only be based upon an "obvious to try" analysis. The additional recitation of a reference (Takeshita) teaching a liposome-based method only furthers the "obvious to try" aspect of this rejection.

The additionally cited Quantin reference also lacks teaching or suggestion of selective transfer into neointimal cells with adenovirus, and in fact teaches away from such a possibility by teachings of expression achieved with an adenoviral vector in non-proliferating cells (myotubes and muscle). Thus the present invention is not achieved by this combination of references, and is also neither taught nor suggested by them. Therefore the present methods are not obvious over this combination of references.

4. **Examiner's position**

Claims 1, 5, 6, 8, 10 and 13-17 stand rejected under 35 U.S.C. § 103 as allegedly obvious over Simons, *et al.* in view of Quantin *et al.* or Willard *et al.* Specifically, the Office Action alleged that the combined teachings of the use of adenoviral vectors as taught by Willard and transfer of antisense oligonucleotides to c-myb oncogene to SMCs to inhibit SMC accumulation would have been obvious to the skilled artisan.

Applicants' Response

Applicants contend that the cited combination of references does not teach a method that selectively targets neointimal cells. As detailed above, and not repeated here, neither Willard nor Quantin teach selective targeting of proliferating or neointimal cells. Quantin in fact teaches transfer using adenovirus into non-proliferating cells. Willard merely teaches localization via infusion into a site in the artery wall by physical placement of the vector. Simons uses a direct DNA uptake method wherein the antisense oligonucleotide is directly applied to the artery wall in the absence of any transfer composition, such as a liposome or an adenovirus.

Thus, the combination of references does not teach the selective transfer into neointimal cells via adenoviruses and thus does not render the present selective transfer and expression method using adenoviruses obvious.

As a separate note, applicants point out, referring back to the rejection under 35 U.S.C. § 112, first paragraph, that Simons demonstrates that the skilled artisan can design and make antisense constructs that achieve inhibition of arterial smooth muscle cells. The Examiner has acknowledged this achievement in the citation of this reference.

5. **Examiner's position**

Claims 2-4 and 9 stand rejected under 35 U.S.C. § 103 as allegedly obvious over Takeshita *et al.* (abstract 3179) in view of Quantin *et al.* or Willard *et al.* as applied to claims 1, 5, 6, 8, 10 and 15-17 and further in view of Takeshita *et al.* (abstract 0903). The August 4, 1996 Office Action alleged its previous rejection that although Takeshita (3179) does not indicate that the transfection was carried out after the time periods required in the claims, the ordinarily skilled artisan would have been motivated to transfect during a period of 5-15 days post-transfection since Takeshita (0903) disclose that this period results in the highest transfection efficiencies, due to increased cell proliferation, and thus the claimed invention would have been obvious.

Applicants' Response

Applicants contend that the combination of Takeshita *et al.* (abstract 3179) in view of Quantin *et al.* or Willard *et al.* does not teach or suggest use of recombinant adenoviral vectors to selectively transfer DNA into neointimal cells, as elaborated fully in (3) above and further applied here. Applicants have fully argued above that it has not been taught by the recited art that adenoviral gene transfer is affected generally one way or the other by the status of the target cell as proliferating or non-proliferating. Applicants cited several references that support this position.

Takeshita (0903) does not cure this deficiency in the combination of references by reciting that a particular time period results in the highest transfection efficiencies in cationic liposome-mediated methods.

In addition, applicants point out that there is a lack of a link between liposome-mediated transfer, which transfer is taught in the two Takeshita references, and adenovirus-mediated transfer, which transfer is herein claimed. The two transfer methods cannot be considered equivalent, since they present to a cell two different compositions. No references or scientific principles have been provided by the Examiner to support that the properties of the two methods

are interrelated in this manner. Therefore, there is no correlation in the record between the two transfer methods that teach one to apply parameters relevant to a liposome transfer method to an adenovirus transfer method.

Conclusion

In summary, the present invention is fully enabled by the specification, particularly in light of the prior art and the skill of the artisan as well as the guidance provided by the specification, to make adenoviral constructs encoding a protein or antisense RNA of choice and use them in the present method to express the protein or antisense RNA in neointimal cells. Furthermore, applicants have enabled use of these methods in human by their description of how to perform the methods in human or any other mammal along with the demonstration in an animal model accepted by many respected scientists in the field. These methods are not anticipated by a reference that does not teach or demonstrate expression in neointimal cells, nor are these methods rendered obvious by a combination of the alleged anticipatory reference with references regarding characteristics of liposome-mediated transfer of nucleic acids.

For the foregoing reasons, it is submitted that the Examiner's rejection of Claims 1-21 was erroneous. Reversal of the Examiner's decision is therefore respectfully requested.

A check in the amount of $1,040.00 is enclosed. Additionally, the Commissioner is hereby authorized to charge $150.00 to Deposit Account No. 14-0629. Thus a total of $1190.00 ($290.00 for the Appeal Brief and $900.00 for the Extension of Time) is submitted. This amount

is believed to be correct; however, the Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

Elizabeth Selby

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I hereby certify that this Appeal Brief is being deposited with the United States Postal Service as first class mail in an envelope addressed to:

Assistant Commissioner of Patents
Washington, D.C. 20231

on this 11th day of June, 1996.

Elizabeth Selby
Elizabeth Selby

6-11-96
Date

APPENDIX A

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APPENDIX B

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Hybritech Inc. v. Monoclonal Antibodies, Inc. 231 USPQ 81, 94 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987)

APPENDIX C

References Relied Upon

Biro, *et al. Proc. Natl. Acad. Sci.* USA 90:654-658 (1993)

Simons, *et al. Nature* 359:67-70 (1992)

Speir, *et al. Circulation* 86:538-547 (1992) (PCNA)

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Guzman, *et al, Circulation Research* 73: 1202-1207 (1993)

Huang, S. *et al., J. Virol.* 69(4):2257-2263 (1995)

APPENDIX D

Appealed Claims

1. A method of selectively expressing DNA in neointimal cells in an injured blood vessel of a subject comprising administering a replication-deficient recombinant adenovirus, which functionally encodes the DNA, to the blood vessel at the site of injury, such that the adenovirus remains at the site of injury for a time sufficient for the adenovirus to selectively infect neointimal cells and thereby selectively express the DNA in neointimal cells.

2. The method of Claim 1, wherein the administration step is performed at least about 4 days after the blood vessel is injured.

3. The method of Claim 1, wherein the administration step is performed at least about 7 days after the blood vessel is injured.

4. The method of Claim 1, wherein the administration step is performed at least about 12 days after the blood vessel is injured.

5. The method of Claim 1, wherein the time the adenovirus remains at the site of injury is from about 15 minutes to about 60 minutes.

6. The method of Claim 1, wherein the DNA encodes a protein.

7. The method of Claim 1, wherein the DNA encodes an antisense ribonucleic acid.

8. A method of treating restenosis in an injured blood vessel of a subject comprising administering to the blood vessel a replication-deficient recombinant adenovirus which

functionally encodes a DNA which can decrease the proliferation of neointimal cells, such that the adenovirus remains at the site of injury for a time sufficient for the adenovirus to selectively infect and express the DNA in neointimal cells, thereby decreasing or inhibiting the proliferation of neointimal cells and treating restenosis.

9. The method of Claim 8, wherein the administration step is performed at least about 4 days after the blood vessel is injured.

10. The method of Claim 8, wherein the DNA encodes a protein.

11. The method of Claim 8, wherein the DNA encodes an antisense ribonucleic acid.

12. The method of Claim 10, wherein the protein is selected from the group consisting of herpes simplex thymidine kinase, dominant negative ras gene product and nitric oxide synthase.

13. The method of Claim 11, wherein the antisense ribonucleic acid is derived from the group consisting of c-myc, c-myb, CDC2 and PCNA.

14. The method of Claim 8, wherein the DNA is cytotoxic to the neointimal cells.

15. A method of decreasing neointimal cell proliferation in an injured blood vessel of a subject comprising administering to the blood vessel a replication-deficient recombinant adenovirus which functionally encodes a DNA which can decrease the proliferation of neointimal cells, such that the adenovirus remains at the site of injury for a time sufficient for the adenovirus to selectively infect and express the DNA in neointimal cells, thereby decreasing the proliferation of neointimal cells.

16. A method of treating primary atherosclerosis in a blood vessel of a subject comprising decreasing neointimal cell proliferation by the method of Claim 4.

17. A method of screening DNA for the ability to inhibit or decrease proliferation of or to have cytotoxic effects on neointimal cells comprising:

a. administering to an injured blood vessel in a subject at the site of injury a replication-deficient adenovirus which functionally encodes the DNA, for a time sufficient for the adenovirus to selectively infect neointimal cells; and

b. detecting inhibition or decrease of proliferation of or toxicity to the neointimal cells, such inhibition or toxicity indicating a DNA having the ability to inhibit or decrease proliferation of or to have cytotoxic effects on neointimal cells.

18. A method of reducing neointimal cell proliferation in an injured blood vessel of a subject comprising administering to the blood vessel (1) a replication-deficient recombinant adenovirus which functionally encodes herpes simplex virus thymidine kinase, such that the adenovirus remains at the site of injury for a time sufficient for the adenovirus to selectively infect neointimal cells, and (2) an effective amount of ganciclovir, thereby reducing the proliferation of neointimal cells.

19. The method of Claim 18, wherein the replication-deficient recombinant adenovirus is administered at any point within about 1 day prior to injury to about 15 days after injury to the blood vessel.

20. The method of Claim 19, wherein the replication-deficient recombinant adenovirus is administered at any point within about 0 to about 7 days after injury to the blood vessel.

21. The method of Claim 18, wherein the ganciclovir is administered in a series of individual doses.

Response Under 37 CFR §1.116
Expedited Procedure
Group 1804

ATTORNEY DOCKET NO. 1414.087
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

FINKEL et al.

Serial No. 08/136,113

Filed: October 13, 1993

For: "EFFICIENT AND SELECTIVE ADENOVIRAL-MEDIATED GENE TRANSFER INTO VASCULAR NEOINTIMA"

Group Art Unit: 1804

Examiner: Railey, J.

DECLARATION

BOX AF
Assistant Commissioner for Patents
Washington, D.C. 20231

NEEDLE & ROSENBERG, P.C.
Suite 1200, The Candler Building
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Atlanta, Georgia 30303-1811

November 3, 1995

I, Toren Finkel, M.D., Ph.D., declare as follows:

1. I am the Toren Finkel named as an inventor in the above-identified application.

2. I have worked in the field of adenoviral-mediated gene transfer for 3 years and have authored about 4 papers in this area of study.

APPENDIX E

3. The proteins and antisense RNA molecules included in claims to methods for treating restenosis in the above-identified application, *i.e.*, herpes simplex virus thymidine kinase, dominant negative ras gene product, nitric oxide synthase (proteins), and c-myc, c-myb, CDC2 and PCNA (antisense RNAs), are known in the art to be cytotoxic or inhibitory to cell proliferation when transfected into cells.

4. Because the above-named proteins and antisense RNAs are known to be cytotoxic or inhibitory to cell proliferation, it is more likely than not that when the genes encoding these molecules are transfected into neointimal cells, they will be cytotoxic or cause a decrease in proliferation of neointimal cells.

5. Restenosis is known to be caused by injury to blood vessels wherein medial smooth muscle cells are activated, begin to migrate, and proliferate to form a neointima.

6. Because restenosis is caused by the proliferation of neointimal cells, causing a decrease or inhibition of neointimal cells is reasonably expected to treat, *i.e.*, prevent or decrease the severity of, restenosis.

7. Therefore, it is credible that when the genes encoding herpes simplex virus thymidine kinase, dominant negative ras gene product, nitric oxide synthase (proteins), and c-myc, c-myb, CDC2 and PCNA (antisense RNAs) are transfected into neointimal cells, they will treat, *i.e.*, prevent or decrease the severity of, restenosis.

8. The conclusion in (7) above is further based upon the example provided in the application wherein the treatment including selective expression of thymidine kinase in neointimal cells in combination

with treatment with ganciolovir was shown to be effective in treating restenosis in the rat corotid artery injury model.

9. Based upon this same reasoning and example, it is also credible that other nucleic acids whose gene products are cytotoxic or cause a decrease in proliferation of neointimal cells will work in the present method to treat restenosis.

10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, imprisonment, or both, under U.S.C. Title 18, § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

11/6/95

Toren Finkel — date

1809_1.WPD